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Regulation of the phosphorylation status of rice retinoblastoma-related protein by PP2A phosphatase

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The retinoblastoma protein (pRB), a nuclear phosphoprotein, is a general regulator of cell proliferation. The ability of pRB and pRB-related proteins to inhibit cellular proliferation is counterbalanced by the action of Cdks (Nakagami et al. 2002). Sixteen potential sites for Cdk-mediated phosphorylation (Ser/Thr-Pro motifs) exist in pRB, and twelve of these sites have been shown to be phosphorylated *in vivo* (Nakagami et al. 2002). In quiescent and early G1 cells, pRB exists in a predominantly unphosphorylated state. As cells progress towards S phase, pRB becomes phosphorylated. Inactivation of pRB by phosphorylation leads to the dissociation and activation of E2F, allowing the expression of many genes required for cell cycle progression and S phase entry (Inoue et al. 2007; Poznic 2009). The phosphorylated state of pRB is accumulating till the end of mitosis when pRB is dephosphorylated by protein phosphatases (Nakagami et al. 2002; Poznic 2009).

Two retinoblastoma-related (RBR) genes have been found in rice, OsRBR1 and OsRBR2. OsRBR2 is expressed mainly in differentiated cells, but the function of the OsRBR1 gene may be related to cell division or cell cycle progression (Lendvai et al. 2007).

Protein phosphatase 2A (PP2A) is a family of serine-threonine phosphatases implicated in the control of a diverse array of cellular processes. The PP2A core enzyme consists of a catalytic C subunit and a structural A subunit. The AC dimer recruits a third regulatory B subunit that has been predicted to dictate the substrate specificity and function of the PP2A heterotrimeric complex. Four unrelated families of B subunits have been identified in mammals (Groves et al. 1999; Yan and Mumby1999; Janssens and Goris 2001; Cicchillitti et al. 2003). In plants B subunits are also important for cellular localization and substrate specificity. B subunits are classified into at least three distinct groups: B, B' and B'', based on molecular weight and domains (Janssens and Goris 2001). The PP2A was reported to be implicated in the dephosphorylation of RBRs, particularly upon oxidative stress (Cicchillitti 2003). A PP2A regulatory subunit (PR70) was also shown to associate with hyperphosphorylated pRb and mediate its dephosphorylation (Groves et al. 1999).

Yeast-two hybrid interaction results show that the OsPP2A B'' regulatory subunit is a strong interactor of OsRBR1, but has no detectable association with OsRBR2.

All the proteins in the RB family can be divided into three regions: the N-terminal region, the pocket domain (include A, B domain and the spacer between A and B) and the C-terminal region. OsPP2A B'' interacts strongly with the OsRBR1 protein minus the C-terminal version, even more strongly than with the full length of OsRBR1, but does not interact with any version of the protein which has some part of pocket domain missing. In addition, there was no interaction between OsRBR1 and EF-hand truncated OsPP2A B'' protein. It was demonstrated that the interaction between the OsRBR1 and OsPP2A B'' proteins needs an intact pocket domain of the RBR protein and the presence of the EF-hands domains on the B'' regulator subunit.

We used OsPP2A B'' regulatory subunit as a bait to screen the interactor from rice suspension cells and leaves cDNA libraries, but then switched to the OsPP2A B'' one-EF-hand minus version because of the self-activation of the full length B'' subunit. We got 30 more interesting interactors of B'' subunits by screen, which we will use in following research.

We have constructed clone encoding the His_6 and GST-tagged OsRBR1, OsRBR2 and OsPP2A B'' proteins using pET-28a and pGEX vectors. Recently, we verified the binding of recombinant His-tagged OsRBR1 protein to GST-PP2A B'' demonstrated that this binding increase in the presence of Ca^{2+}.

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